ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 1, pp: (117-126), Month: January - March 2019, Available at: www.researchpublish.com

MASSMULTIPLICATION OF *TRICHODERMA HARZIANUM* USING AGRICULTURAL WASTE AS A SUBSTRATE FOR AGAINST *FUSARIUM OXYSPORUM* IN MAIZE PLANTS (*ZEA MAYS L.*)

[1]Pavithra. R, [2*]Lalitha.S

[1,2] Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar, Salem-11.
Tamil Nadu, India
E-mail: *pavirambot@gmail.com*

Abstract: **Applying chemical fungicides to control soil-born fungi may lead to environmental pollution. In recent years, microorganisms to control plant pathogens, to improve yield and quality of crop. Fungal species belonging to the genus *Trichoderma* act as biological agent. Genus *Trichoderma* effective biocontrol agent against fungal, bacterial pathogen. The fungal isolates *Trichoderma harzianum* (Tr01) and *F. oxysporum* (Fu04) was also identified through morphological characterization observed under light microscope (10x×40x). The isolates (Tr01, Fu04) were confirmed through genetic DNA isolation & PCR analysis. After that pot culture study was conducted to test the antagonistic potential of Tr01 against Fu04. Healthy seeds of maize *(Zea maize L.)* were used in this experiment, They are three different substrates (Rice bran, Black gram bran ,Sugarcane baggase)and soil, water are used in the following ratio of 1:1:2. Nine treatments are T_1 Control, T_2 *Trichoderma harzianum*, T_3 *Fusarium oxysporum* , T_4*Trichoderma harzianum* + Ricebran, T_5*Trichoderma harzianum* +Rice bran + *Fusaium oxysporum*,T_6 *Trichoderma harzianum* +Blackgram bran , T_7 *Trichoderma harzianum* + Blackgram bran +*Fusarium oxysporum*, T_8*Trichoderma harzianum*+Sugarcane baggase ,T_9*Trichoderma harzianum* + Sugarcane baggase + *Fusariumoxysporum*.In pot culture after 45 DAI highest plant length for T_2 (80cm), highest fresh weight for T_6 (0.070), highest dry weight for T_2 (0.020) ,highest chlorophyll content T_4(9.8695).**

Keywords: **Biocontrol, *Trichoderma harzianum*, *Fusarium oxysporum*, Antagonistic effects, organic substrates.**

I. INTRODUCTION

Trichoderma species are one of the most important bio-control agents and most frequently isolated from soil and root zone of plants. *Trichoderma* strains are antagonistic to some plant pathogenic fungi because they have the ability to suppress the diseases. *Trichoderma* uses several mechanisms such as antibiosis, myco-parasitism and competition for nutrients and space, and is also able to promote growth and development of plant and induce the defense response of plants. Several strains of *Trichoderma* spp. have been found to be effective as biocontrol agents of various soil-borne plant pathogenic fungi such as *Fusarium, Pythium, Rhizoctonia* and *Sclerotium*. (Reena *et al*., 2013)

Trichoderma spp. grow in different inorganic inert materials and organic substrates are used as carriers or delivery media during formulation. Some of the commonly used locally available substrates are agro-industrial cellulosic wastes (wheat straw, paddy straw, shelled maize cob, saw dust, paper waste, vegetable and fruit waste, coffee waste, tea waste and

sugarcane bagasse), organic manures (farm yard manure, neem cake, vermiculite and spent mushroom compost) and cereal bran (wheat, grain of sorghum, wheat and pulse and rice bran, (Yadav, 2012; Babu and Pallavi, 2013) were also being used which support better shelf life of the *Trichoderma* spp. as well its better performances in management of collar rot disease of cowpea with promotion of growth as well. *Trichoderma* is of immense importance not only to agriculture and its crops but also to the environment as it does not accumulate in the food chain and thus does no harm to the plants, animals and humans (Perveen and Bokhari, 2012; Reena et al., 2013). The cost of these raw materials for commercial production of biocontrol agents is one of the major limitations behind the restricted use. To overcome the cost limitation, many researchers have successfully used substrates like composted coir pith, coffee wastes and poultry manures, neem cake, coir pith, farmyard manure (FYM) and decomposed coffee pulp (Saju *et al*., 2002)

In this study in vitro condition *Trichoderma harzianum* grow in different substrates to using formulate based soil in plant growth.

II. MATERIALS AND METHODS

Collection of soil

The soil samples were collected from different locations of Agricultural land, Alagapuram , places from Salem District, Tamilnadu .The soil collected from 10-12 cm depth near root zone of rhizosphere soil of healthy plants.

PDA medium

Potato dextrose agar (PDA) Medium were used to culture fungi from each soil sample , potato dextrose agar (PDA) .This culture medium was also used liquid from without agar added for dilution preparation.

Serial dilution method (Ben David and Davidson, 2014)

A serial dilution plating method was followed by Ben David and Davidson, 2014.

Isolation of microorganisms (*Trichoderma harzianum*) from soil

Collected soil sample diluted in distilled water. Isolation was made through dilution plate technique. 20 ml warm melted PDA medium was poured in each sterile petriplate.1 ml of diluted soil sample (10^{-4}) was placed at the center of PDA and spreaded. Four petridishes were inoculated with 1ml of each diluted sample. The inoculated PDA plates were incubated for 7-10 days at room temperature (25 ± 1^0C). The colonies grown out on PDA were recorded after 3-5 days of incubation.Culture was transferring a small colony to a new Petridis on the basis of color and morphology of the colony. Further cultures were made for purification.

Identification of the isolates

Trichoderma harzianum isolate characterized by a rapid growth rate in culture and by the production of numerous spores (conidia) with varying shades of green, microscopically observe for colony growth rate, colony color, reverse color, colony edge, mycelial form, conidiophore branching, conidial color were selected and microscopic observation available in literature (Shahid *et al.,* 2013) .

Isolation of pathogen

The infected tomato leaf tissue of the collar region of the plant was collected and repeatly washed in fresh water and surface was sterilized by three times washing in distilled water. Then the species of infected tissue were placed on PDA medium was inoculated at 22 ± 2^0C 7 days. After incubation, white mycelia formed the pathogen was purified and multiplied subsequently through hyphal tip culture on PDA, for preparation of inoculums.

Identification of pathogen

Fusarium oxysporum just based on colony appearance and morphological characteristics of *Fusarium oxysporum* produced white to pale violet or pink pigmentation, for naked eyes and microscopically.

Antagonistic activity (Skidmore and Dickinson 1976)

The antagonistic activity of *Trichoderma harizanum* against *Fusarium oxysporum* and were studied in dual culture method. PDA plates were inoculated with 5 mm disc from 5 days old culture of the plant pathogens in the center of the plate. There 5 mm disc of the *Trichoderma harzianum* isolate was placed on the periphery of the Petri plate. Another

method for PDA plates were inoculated by placing a 5mm diameter mycelial disc of the *Trichoderma harzianum* on one side obtained from a 5 days old culture on PDA. A similar disc of the *Fusarium oxyporum* obtained from the growing edge of 5 days old culture on PDA was placed on the opposite side of the *Trichoderma spp*. The inoculated plates were incubated at (28±2^{0}c)for room temperature for 7 days .The contacts zones of two colonies were observed under the light microscope for any interaction between the fungi and radial mycelia growth of the test pathogen was determined by measuring radial growth.

Genomic DNA Isolation from Fungi

Procedure

1.To a nuclease free 1.5ml centrifuge tube, add 200µl fungi cells. Centrifuge for 5min at 3000Xg and resuspend the cell pellet in 200µl PBS.

2. Reconstituted proteinase K. Mix immediately and incubate for 10min at 70˚C.

3. Add 100µl isopropanol and mix well.

4. Assemble one high pure filter tube into one collection tube. Pipet the liquid sample into the upper buffer reservoir of the filter tube. Centrifuge 1min at 8000Xg.

5. Remove the filter tube from the collection tube. Discard the flow through liquid and the collection tube.

6. Add 500µl inhibitor removal buffer to the upper reservoir of the filter tube and centrifuge 1min at 8000Xg.

7. Remove the filter tube form the collection tube. Discard the flow through liquid, the collection tube. Assemble the filter tube with a new collection tube. Add 500µl wash buffer to the upper reservoir of the filter tube. Centrifuge 1min at 8000Xg and discard the flow through

8. After discarding the flow through liquid, centrifuge the entire high pure assembly for 1min at full speed. Discard the collection tube. 9. Insert filter tube into a clean, sterile 1.5ml micro centrifuge tube. 10. Add 200µl prewarmed elution buffer to the upper reservoir of the filter tube. Centrifuge the tube for 1min at 8000Xg. The micro centrifuge contains eluted DNA and stored at 20˚C.

Electrophoresis methods

- ❖ Agarose Gel Electrophoresis Requirements
- ❖ Balance
- ❖ Gel Apparatus
- ❖ Power Pack
- ❖ Micropipettes
- ❖ Microwave oven
- ❖ Gel Documentation system

Buffers and Reagents

TAE Buffer (50X) Tris base - 242 g Glacial acetic acid - 57.1ml EDTA 0.5M (pH 8.0) - 100ml TBE Buffer (5X) Tris base - 54.0 g Boric acid - 27.5 g EDTA 0.5M (pH 8.0) - 20ml ,54.0g of tris base and 27.5g of boric acid were dissolved in 800ml of distilled water. 20ml of EDTA (pH 8.0) was added to this and volume was made up to 1000ml with distilled water. Autoclaved and stored at room temperature.

6X DNA Loading Buffer Bromophenol Blue - 25mg Xylene cyanol FF - 25mg Glycerol - 3ml 25 mg bromophenol blue and 25 mg xylene cyanol were dissolved in 5ml of distilled water. 3 ml of glycerol was added and volume made up to 10ml. store at 4˚C.

Ethidium bromide stock Dissolve 10mg of Ethidium bromide in 1ml of water. Final concentration 0.5µg/ml

Procedure

1. Seal the edges of a clean, dry glass plate.

2. Prepare sufficient electrophoresis buffer (usually 1x TAE or 0.5X TBE) to fill the electrophoresis tank. Add 0.5g of powdered Agarose to 50ml of the buffer. Heat the slurry in microwave oven until the Agarose dissolves.

3. Cool the solution to 60˚C and add Ethidium bromide and mix thoroughly.

4. Position the comb 0.5-1.0mm above the plate so that a complete well is formed when the Agarose is added.

5. Pour the warm Agarose solution into the mold. The gel should be between 3mm and 5mm thick. Check to see that there are no air bubbles under or between the teeth of the comb.

6. After the gel is completely set, carefully remove the comb and the tape and mount gel in the electrophoresis tank.

7. Add just enough electrophoresis buffer to cover the gel to a depth of about 1mm.

8. Mix the samples of DNA with the desired gel- loading buffer. Slowly load the mixture into the slots of submerged gel.

9. Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the anode.

10. Turn off the electric current and remove the leads and lid from the gel tank. The gel was examined under ultra violet light and photographed.

Waste Disposal Procedures

Place waste Agarose that is contaminated with Ethidium Bromide, in a sealable, liquid-tight, labeled waste container.

Polymerase Chain Reaction

Scope PCR is an in vitro enzymatic process by which a specific region of DNA is synthesized into many copies, and this was discovered by Kary Mullis. This molecular photocopying process involves heating and cooling of samples in a machine called thermal cycler in the presence of oligonucleotide primers, dNTPs and heat stable enzyme called Taq Polymerase in a cycle pattern over about 30 cycles. During each cycle, a copy of target DNA sequence is generated for every molecule containing the target sequence. After about 30 cycles, a billion copes of the target region on the DNA template have been generated. This PCR product, also called as amp icon.

Requirements

Gloves, PCR Thermal cycler , Pipettes (1-10 µl, 5-50 µl, 20-200 µl, and 100-1000 µl) aerosol, barrier pipette tips ,PCR tubes (0.2 ml or 0.5 ml) , Master mix tubes (1.5 ml micro centrifuge tubes) 3.3. Buffers and Reagents, DNA Template, Buffer,dNTPs.Taq Polymerase, Primers, HPLC water

Procedure

1. Remove the reagents and DNA from the freezer and allow them to thaw on ice. You can let them thaw at room temperature, but be sure to immediately put them back on ice once thawed. 2. Using sterile pipette tips, add nuclease-free water to the master mix tube (125µl) as follows Components µl/rxn µl all rxn (Master mix) Double distilled water 18.3µl 91.5µl Taq DNA polymerase buffer (10x) 2.5µl 12.5µl dNTP mix (2mM) 2.5µl 12.5µl Forward Primers (10µM) 0.5µl 2.5µl Reverse Primers (10µM) 0.5µl 2.5µl Template DNA 0.5µl - Taq DNA polymerase enzyme (5U/µl) 0.2µl 1.0µl Total Reaction Volume 25.0µl 122.5µl.

3. Take five 0.2ml or 0.5 ml PCR tubes and label appropriately.

4. Briefly vortex the master mix or mix thoroughly by tapping and aliquot 24.5 µl into each of the five reaction tubes.

5. Add 0.5 µl of each DNA sample to the respective PCR tube. Remember, the negative control receives no DNA.

6. Cap the tubes, mix the contents by flicking with your finger, and then briefly (~10 seconds) centrifuge the tubes to concentrate the reaction mix at the bottom of the tube.

7. Place the tubes in the PCR the rmalcycler, close the lid, and start the programme. Thermalcycler programme Initial denaturation - 94ºC for 5 minutes Cycle denaturation - 94ºC for 45 Seconds Annealing - 55ºC for 45 Seconds Extension - 72ºC for 1 min Go to step 2 for 34 cycles Final Extension - 72ºC for 5 minutes Hold 22ºC forever End programme 8. Stop the programme and Collect the tubes. 9. Resolve the PCR product onto 1% agarose gel and observe.

Collection of substrates

Substrates were collected from Salem LI Bazar, from Rice bran, Black gram bran, Sugarcane bagasse and sorghum grains.

Collection of seeds

Maize seeds are collected from agricultural office (seelanayakanpatty) in Salem.

Variety of seed

Maize (Zea maize)– shivani.

Pot culture experiments

Altogether 3 soil based substrates along with a control were explored in the

Experiment stated below

- Rice bran + soil + water (1:1:2)
- Blackgram bran + soil + water (1:1:2)
- Sugarcane baggase + soil + water (1:1:2)

Treatement

- Control
- *Trichoderma*
- *Fusarium*
- *Trichoderma* + rice bran
- *Trichoderma* + rice bran + *Fuarium*
- *Trichoderma* + blackgram bran
- *Trichoderma* + blackgram bran + *Fusarium*
- *Trichoderma* + sugarcane baggase
- *Trichoderma* +sugarcane baggase + *Fusarium*

Sterilization of substrates and inoculation of *Trichoderma* and *Fusarium oxyporum* in multiplication

The requisite amount of materials for each substrate was thoroughly mixed in a1000 ml Erlenmeyar flask and autoclaved at 121^{0}C for 15 minutes for sterilization. The sterilized substrates allowed to cool down and then inoculated with 5 mm dia mycelia disc of 7 days old *Trichoderma* and *Fusarium oxyporum*) culture. Seven discs for each flask were used for inoculation. Inoculated flasks were then incubated at room temperature (25 ±2)^{0}C for 15 days.

Application of *Trichoderma* and pathogen in the soil

The soil was sterilized in autoclave for 15 LPs pressure the sterilized soil was cooled. After the soil was mixed with substrates they are filled up with the pot. Formulated *Trichoderma* were mixed with the soil of each earthen pots of 20 g /kg soil. The treated soil was incubated for 7 days maintaining proper soil moisture. After that, soil was inoculated with sorghum grains colonized by *Fusarium oxysporum* 20g/kg of soil. Inoculated soil was incubated for 7 days maintaining proper soil moisture.

Growth parameter of plant

Plants were harvested at 45 DAI (Day after Inoculation) and separated in to leaves, shoot and root. The plant length was calculated in bio inoculants treated plants and non-inoculants plants.

Estimation of photosynthetic pigments (mg/g/ fr .wt) (Arnon 1949)

Chlorophyll a and b contents were extracted from respective dose of leaves and estimated according to the method of Arnon (1949) and the carotenoids content was determined according to the method of Kirk and Allen (1965).

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 1, pp: (117-126), Month: January - March 2019, Available at: www.researchpublish.com

Extraction

Two hundred mg of fresh young leaves were grind with 10 ml of 80% acetone pestle and morter and spun at 2500g for 10 minutes at 20^0C in centrifuge. The homogenate was re extracted with 80% acetone until the green colour was disappears in the residue and the extract was pooled and made up to 20ml with 80% acetone.

Soil analysis

Soil pH and Electrical Conductivity

The soil samples were dried and stored in a refrigerator at 5^0C for analysis. The pHof the soil samples was measured with ph meter using 1:2:5 soil/wet system (Elico,India) and Electrical Conductivity(EC)was measured using an electrical conductivity bridge (Elico type CM 82.India) and was expressed as dSm^{-1}.

Soil nitrogen

The available nitrogen content in the soil was estimated by the alkaline permanganate method as described by Subbish and Asija(1956).

Soil phosphorus

Soil phosphorus was estimated by the method by the given by (Olsen etal.1954).

Soil potassium

Potassium content of the soil was estimated by the method of (Jackson,1973).

III. RESULTS

Isolation and identification of *Trichoderma*

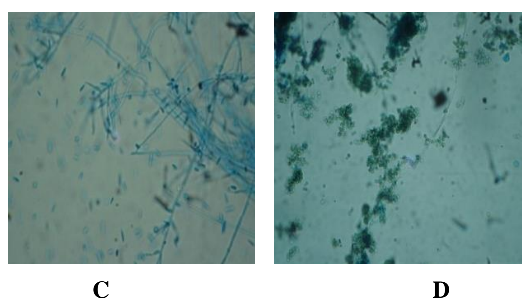
Trichoderma fungal species was isolated from agriculture land soil samples in Alagapuram, Salem District, Tamilnadu, India. Serialy diluted samples were placed on PDA medium colony that produced on green colour. And the sample was named as *Trichoderma* and the culture was maintained in potato dextrose agar (PDA) medium. The fungal species*(Trichoderma*) was identified through morphological characterization of green color conidia was picked observed under the light microscope (10x×40x) and the result was defected in (Fig-1,2)

Isolation and Identification of fungal pathogen (*Fusarium oxysporum)*

The fungal pathogen was isolated from tomato plant infected leaf maintained in Potato dextrose agar medium. Identified through morphological character observed under the light micrope (10x×40x)and the result was defected in (Fig-1,2).

A B

Fig 1: Morphological view - A)*Trichoderma harzianum* B)Plant Pathogen-*Fusarium oxysporum*



C D

Fig 2: Microscopic view (10x×40x) - *C) Trichoderma harzianum D) Fusarium oxysporum*

Antagonistic activity

Trichoderma isolation was screened for their antagonistic potential against different fungal pathogens using dual culture technique. *Trichoderma* species were screened for antifungal activity against *Fusarium spp.* and zone of inhabitation was taken as an indicator of antifungal property in the dial culture method. The isolates tested positive for antifungal activity will be further exploded in for pot and field experiments to study plant growth, yield, and biocontrol ability on maize plant (Fig-3).

Fig 3: Antagonistic activity of dual culture in plate techniques

PCR analysis

The microbial isolates (Tr01) were confirmed through genetic DNA isolation and PCR analysis (Fig-4).

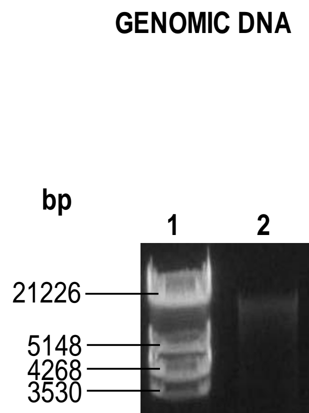


Fig 4: Agarose Gel (1%) showing Lambda DNA / Eco RI Marker & Genomic DNA

Lane 1 – Lambda DNA / Eco RI Marker

Lane 2 – Genomic DNA of PF

Pot culture studies

The result obtained from the study on the effect of four different treatments,T_1(*Trichoderma*+ soil +Water), T_2(*Trichoderma* +Ricebran+ Soil+ Water),T_3(*Trichodema* +Black gram bran) T_4 (*Trichoderma* +Sugarcane baggase + Soil +Water) T_4(*Fusarium*) T_5(Control) management of maize seeding.(Fig-5)

Determination of plant length in (cm)

Plant length was measured in three plants per pot .Each replicates were measured in centimeters (cm) using ruler. Using bioinoculated plants showed highest growth parameterTr01(80cm), the second height for Tr01+Sugarcane baggase (70.6) when compared to that of control (39.4 cm) less pronounced in *Fusarium ohysporum* inoculated plants(38cm). Bio inoculants was highly enhanced the plant length (Fig-6,7,8).

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 1, pp: (117-126), Month: January - March 2019, Available at: www.researchpublish.com

Determination of fresh weight for plants in (g)

The measurement was taken after 45 DAI (Day after Inoculation). They were compared to bio inoculated plants and control. Leaf, Stem and Root fresh weight was measured in three plants per pot .Similarly biocontrol agents *Trichoderma* inoculated plant fresh weight (0.074g) when compared to that of control (0.031 g) and *Fusarium* (0.026 g). Bio inoculants was highly enhanced the plant fresh weight. (Fig-9)

Determination of dry weight for plants in (g)

After recording the fresh weight of plants placed inside of the hot air oven for continuous 3days.After 3 days of incubation the dry weight of plants was recorded and the data obtained were analysis. The fresh and dry weight of plants *Trichoderma* inoculated plants show more dry matter yield when compared to control and *Fusarium* is low dry matter. Similarly biocontrol agents *Trichoderma* + Rice bran inoculated plants dry weight (0.021g), the second dry weight for Trichoderma + sugarcane inoculated plant(0.015g) when compared to that of control 0.07 g) and *Fusarium* (0.04 g)plant pathogen was highly inhibited using bio inoculants. Bioinoculants was highly enhancing the plant dry weight. (Fig-9)

Fig 5: Substrates preparation in conical flask for 15 DAI (Days After Inoculation)

Fig 6: Shoot length of *Zea mays L.*(cm) at 45 DAI (Days after inoculation)

Fig 7: Root length of *Zea mays L.*(cm) at 45 DAI (Days after inoculation)

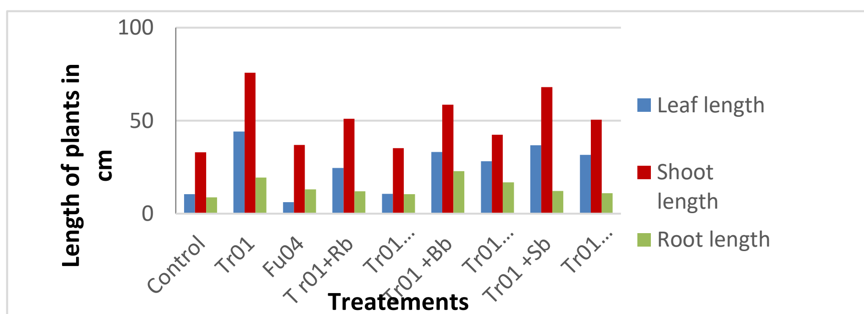


Fig 8: Length of (*Zea mays L.*) Leaf, shoot, and root length of plants at 45 DAI (cm/plant).

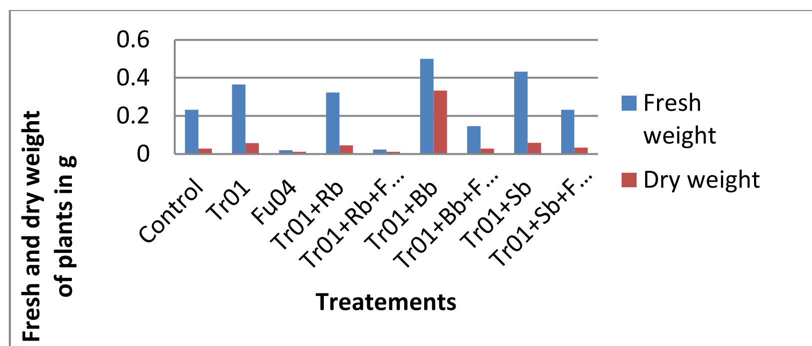


Fig 9: Growth characters of (*Zea mays L.*) Fresh weight and Dry weight of plants in (g)

Determination of chlorophyll (mg/g/fr.wt)

The result showed clearly the effects of *Zea maize* on growth parameter of crop .parameters were investigated in leaf chlorophyll content the total chlorophyll content was ultimately affected and its accumulation was significantly reduced to compared with control, The total chlorophyll content in *Zea maize* was significantly low. The result showed that there was high chlorophyll accumulation in control, *Trichoderma* treated pots . Similarly biocontrol agents *Trichoderma* inoculated plants total chlorophyll content (9.8695) when compared to that of control (4.8726) and *Fusarium* (2.7580).Bioinoculants was highly enhance the plant chlorophyll content. (Fig.10)

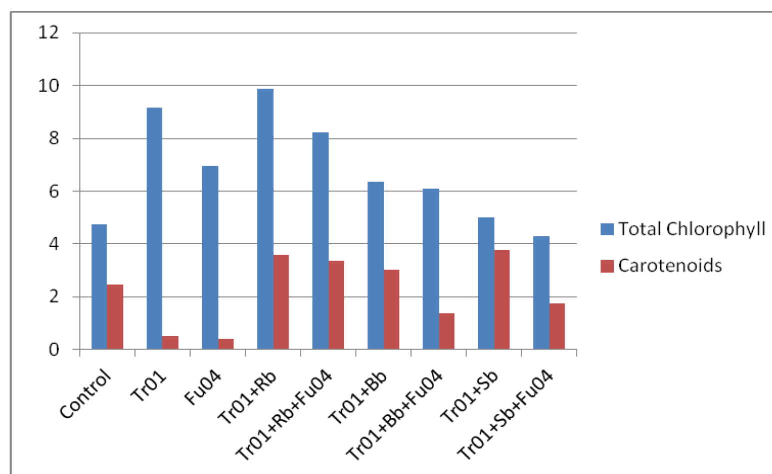


Fig 10: Analysis of total chlorophyll content in (*Zea mays L.*) at 45 DAI (mg/g/fr.wt)

Total nitrogen content and Soil analysis

Soil NPK content was more pronounced in bio inoculated soils and as well as in field trials un inoculated soil had less NPK content, and bioinoculants inoculated soil contain high level nutrients.Soil NPK content was more pronounced in dual inoculated soils and as well as in pots experiment. Uninoculated soil had less NPK content, and Bioinoculants inoculated soil contain high level of nutrients.

IV. DISCUSSION

The present study was carried out in Periyar University Department of botany soil sample collected from Alagapuram in Salem district, Tamilnadu. Plant pathogen were isolated from infected plant material plant material in Agricultural land Alagapuram Salem. The Identified Cultures *Trichoderma harzianum, Fusarium oxysporum* maintained on PDA medium. The morphological characterization of *Trichoderma harzianum* showed light green colour colony appearance. *Trichoderma harzianum* shows antagonistic effects against *Fusarium oxysporum*. (Kucuk and Kivanc,2008). The microbial isolates (Tr01) were confirmed through genetic DNA isolation & PCR analysis. The isolate showed maximum similarity with *Trichoderma species* based on 16S rRNA analysis (Sambrook, J. Fritsch, E. F. and Maniatis, T. 1989).

Pot culturestudies using different substrates Rice bran + soil + water, Black gram bran + soil + water ,Sugarcane baggase + soil + water, prepared in the ratio of 1:1:2. Nine different treatements of *Trichoderma harzianum* based substrates.T_1 (Control), T_2 (*Trichoderma harzianum*),T_3 *(Fusarium oxysporum*),T_4 (*Trichoderma harzianum* + Ricebran),T_5(*Trichoderma harzianum* + Rice bran +*Fusarium oxysporum*),T_6 (*Trichoderma harzianum* + Black gram bran),T_7 (*Trichoderma harzianum* +Blackgram bran + *Fusarium oxysporum*),T_8 (*Trichoderma harzianum* +Sugarcane baggase), T_9 (*Trichoderma harzianum* +Sugarcane baggase +*Fusarium oxysporum*).(Jannatul adan,2013).

The following characteristics were studied with effect of inoculated and uninoculated plant biomass, photosynthetic pigments such as chlorophyll a, b, carotenoids component, soil macro and micronutrients, and total nitrogen content in nine treatments (Tr01) compared with control. (Almas Zaidi *et al*., 2015).

The nine treatement shows after 45 days highest plant length for T_2 (80cm), highest fresh weight for T_6 (0.070), highest dry weight for T_2 (0.020),highest chlorophyll content T_4(9.8695).

REFERENCES

[1] Almas Zaidi, Ees Ahmad, Mohamad Saghir Khan, Saima Saif And Asfa Rizvi. 2015.Role of Plant Growth Promoting Rhizobacteria In Sustainable Production of Vegetables *Currentperspective Saientia Horticulturae*. 193:231-239.

[2] Arnon, OI.1949. Copper Enzyme In Isolated Chloroplasts.1. Polyphenol Oxidase In *Beta Vulgaris .Plant,* 24: 1-15.

[3] Babu KN And Pallavi PN. 2013. Isolation, Identification And Mass Multiplication Of *Trichoderma* An Important Bio-Control Agent. *International Journal Of Pharmacy And Life Sciences*.4(1): 2320-2323.

[4] Bendavid A And Davidson E. 2014. Estimation Method for Seriol Dilution Experiments. *Journalof Microbiological Methods* 214 -221.

[5] Jackson ML. 1973. Soil Chemical Analysis. Prentice Hall f India Pvt, Ltd., Newdelhi.498.

[6] Jannadal Adan MD. 2013. Evalution of Trichoderma Based Biopesticide Formulation For Controlling Damping off Pathogen of Egg Plant And Tomato Seedling.

[7] Kirk JTO And Allen RL. 1965. Dependence of Choloroplast Pigment Synthesis on Protein Synthesis. Effect of Actidione. *Biochemical Biophysical ResearchCommunication***.**21: 523-530.

[8] Kucuk C. Kivanc M. 2008. Isolation of *Trichoderma* Spp. And Determination of Their Antifungal, Biochemical And Physiological Features. Turkey Journal of Biology. 27**:** 247-253.

[9] Olsen SR, Cole CV, Watanabe FS And Dean LA.1954. Estimation of Avoilable Phosphorus In Soil Extraction With Sodium Bicarbonate.U.S.939.

[10] Perveen K And Bokhari NA.2012. Antagonistic Activity Of *Trichoderma* Harzianum And Trichoderma Viride Isolated From Soil of Date Palm Field Against Fusarium Oxysporum. *Afr. J. Microbiol. Res*. 6(13): 3348-3353.

[11] Reena A, Anitha M, Aysha OS, Valli S, Nirmala P, Vinothkumar P.2013. Antagonistic Activity of Trichoderma Viride Isolate On Soil Borne Plant Pathogenic Fungi. *International Journal of Bioassays*; 2:294-297.

[12] Saju KA, Anandaraj M And Sarma YR.2002. On Farm Production Of Trichoderma Harcianum Using Organic Matter. *Indianphytopath***.** 55: 277-281.

[13] Skidmore AM, Dickinson CM.1976. Colony internations and hyphal interference between Saprophytic and phyllophance fungi. *Trans.Br. Mycol. Soc* 66:57-64.

[14] Subbiah BV And Asija GL.1956.A Rabid Procedure For Determination Of Avoilable Nitrogen In Soil .*Curr.Sci*.25: 259-260.

[15] Yadav LS.2012.Antagonistic Activity Of *Trichoderma Sp.* And Evalution Of Various Agro Waste For Mass Production. *Indian Journal Of Plant Science* .1(1):2319-3824.